

REMARKS

In the Office Action dated January 8, 2010, claims 1-2, 13, 15-17, 28 and 30-33 were pending and under consideration. Claims 17 and 32 were objected to for typographical errors. Claims 1-2, 15-17 and 30-33 were rejected as allegedly unpatentable under 35 U.S.C. §103(a) over Gatterman in view of Greiner (*Am. J. Pathol.* 146: 46-55, 1995). Claims 13 and 28 were rejected as allegedly unpatentable over Gatterman in view of Greiner, and further in view of Nomoto et al. (*Clinical Cancer Res.* 8: 481-487, 2002) and Sanchez-Cespedes et al. (*Cancer Research* 61:7015-7019, 2001).

This Response addresses each of the Examiner's rejections and objections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim Objections

Claims 17 and 32 have been amended to correct certain typographical errors. No new matter is introduced. Withdrawal of the objections is respectfully requested.

35 U.S.C. §103(a) Rejections

Claims 1-2, 15-17 and 30-33 were rejected as allegedly unpatentable under 35 U.S.C. §103(a) over Gatterman (*Leukemia Res.* 24: 141-151, 1999) in view of Greiner (*Am. J. Pathol.* 146: 46-55, 1995). Claims 13 and 28 were rejected as allegedly unpatentable over Gatterman in view of Greiner, and further in view of Nomoto et al. (*Clinical Cancer Res.* 8: 481-487, 2002) and Sanchez-Cespedes et al. (*Cancer Research* 61:7015-7019, 2001).

The Examiner alleges that Gatterman teaches clonality of mitochondrial DNA (mtDNA) sequences and the use of TGGB to identify mitochondrial DNA mutations based on colocalization resulting from the existence of sequence identity.

Applicants wish to draw the Examiner's attention to the fact that although the TGGE analysis is used, the objective of Gatterman's analysis is to detect the existence of heteroplasmy of mtDNA. This is a different issue from detecting the existence of clonality of daughter cells which exhibit a common acquired mitochondrial mutation. Heteroplasmy is the presence of a mixture of more than one type of an organelle genome within a cell or individual. The severity of mitochondrial diseases is linked to the proportion in mitochondria in a single cell which have incorporated a mutation versus the proportion of mitochondria, within the same cell, which have not acquired the subject mutation. Once a sufficiently high proportion of mitochondria within a single cell exhibit a mutation, disease symptoms become evident. This is demonstrated in Figure 2 of Gatterman where the mutation is present in the progenitor cell and the issue is one of the random segregation between daughter cells of the *proportion* of mitochondria exhibiting the subject mutation. Accordingly, the pattern which the authors of Gatterman look for in the TGGE results is in fact the co-existence of a proportion of wild-type DNA and a proportion of mutant DNA in a single sample. The cells which were screened were cells which were known to be bone marrow cells of patients with sideroblastic anaemia.

The colocalization which is discussed in the present application, being the colocalization of a population of mitochondrial DNA exhibiting a common acquired mutation, which is acquired at times when the descendants of an ancestral cell divide to form new daughter cells, and which is therefore indicative of a clonal population of daughter cells, is not taught in Gatterman. Gatterman teaches the occurrence of a mutation in a progenitor cell which is carried

through to all daughter populations and all ongoing generations of cells, the only issue being the random segregation of the mutated mitochondria within a given cell relative to non-mutated mitochondria, with a high percentage of mutated mitochondria being associated with the onset of disease. The present invention, however, does not focus on the issue of comparing mutated versus non-mutated mitochondrial DNA within a single cell in order to analyze the existence and extent of heteroplasmy; but rather, is merely directed to detecting the expansion of a clonal population of cells which exhibit a common acquired mutation. These cells may themselves, in subsequent generations, lead to the development of clonal populations as further mutations are acquired. This is not the case in the context of the analysis being performed by Gatterman, where the condition is characterized by the occurrence of a mitochondrial mutation in a progenitor cell and which is maintained in all subsequent daughter cells.

Accordingly, the authors of the Gatterman reference were not screening to identify the existence of clonal populations of cells, but were using TGGE to analyze a known dysplastic population of cells to determine their relative heteroplasmy with respect to mitochondrial DNA mutations. Their analyses still went further to indicate that the subject mutations are causative of disease and are present throughout the disease and defined by specific nucleotide changes which were determined by sequencing. In contrast, the method of the present invention does not involve an analysis of the nature of the specific mutation as an adjunct to the analysis of the potential impact of the relative heteroplasmy which is identified by the TGGE method in Gatterman.

In fact, the Examiner's attention is directed to the text which appears in the second paragraph on page 148 of Gatterman, where it is indicated that where a subclone is generated within a pool of clonogenic cells, that subclone would probably not be detectable via the specific

method taught in Gatterman. This is the very type of subclone that would be detected by the method of the present invention. More particularly, Figure 4A of Gatterman depicts this type of subclone, which is admitted by Gatterman as not detectable by the method taught by Gatterman which is focused on analysis of heteroplasmy. The situation in Figure 4B where all cells carry the stable mutant would be detectable, and the detection is premised on that the cell containing the mutation becomes dominant and exhibits a growth advantage. The authors of Gatterman in fact state that the mutations that they have identified were likely present in the haemopoietic stem cell, and therefore are genomic mutations carried through to all daughter cells, unlike the mutations which are detected by the present invention, namely, mutations acquired from one generation to the next and are detectable by the method of the invention where clonal expansion of a daughter cell population has occurred.

Therefore, Applicants respectfully submit that the Examiner's reliance on Gatterman as a primary reference against the claimed invention is misplaced.

In relation to Greiner, Applicants reassert that Greiner is directed to looking for existence of mutations at the germline level albeit via a colocalization technique. Greiner is not directed to analyzing mitochondrial DNA. As submitted in Applicants' previous Response, Greiner relates to an analysis of the clonality of tumor cells in leukemia/lymphoma patients using PCR and denaturing gradient gel electrophoresis, directed to analyzing T cell receptor (TCR) γ gene rearrangements. The type of change involved respecting the genes encoding the T cell receptor is unique in that the change takes the form of the rearrangement of sections of the germline DNA. However, the presently claimed invention is directed to analyzing DNA regions in which acquired mutations occur *at the time that the descendants of an ancestral cell divide to form new daughter cells*. The rearrangements that occur to the T cell receptor DNA do not occur

at this time. Rather, only after a cell has become committed to the T cell or B cell lineage does it undergo germline DNA rearrangement and thereafter all daughter cells express *the same* rearrangement pattern. Therefore, Applicants respectfully submit that the TCR γ gene rearrangement of Greiner is an entirely different type of cellular event, and is not relevant to the assessing the invention as claimed.

With regard to Nomoto, this article teaches the identification of a specific mutation to detect cancer. This reference is not relevant to the present invention because it is based on the existence of a specific nucleotide substitution as diagnostic of a particular disease, this being hepatocellular carcinoma. The approach taught in Nomoto requires knowledge of the relevant DNA sequence and mutation, which is not required in the context of the method of the present invention. Still further, the invention as presently claimed is directed to non-neoplastic cells, in contrast to cancer cells taught in Nomoto.

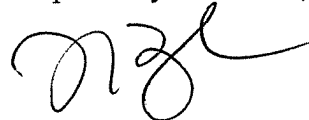
Applicants further respectfully submit that the methodology of the present invention is highly sensitive and specific, being capable of detecting and differentiating mutations at different locations within the same region (D-loop), which represents a significant advantage over the prior art, including the method disclosed in Gatterman, which admittedly would not detect a clonal subpopulation cells exhibiting a mitochondrial mutation.

In summation, Applicants respectfully submit that the combination of the cited references do not render the present invention obvious. Therefore, the rejection under 35 U.S.C. §103(a) based on the combination of the cited references is overcome, and withdrawal thereof is respectfully requested.

Conclusion

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

A handwritten signature in black ink, appearing to be 'XZ' followed by a stylized flourish.

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